COMPARISON OF A SINGLE SPOT CONCENTRATED SPUTUM AFB SMEAR VERSUS 2 DIRECT SPUTUM AFB SMEARS DONE REGULARLY IN REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME.

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Madras Medical College & Rajiv Gandhi Government General Hospital

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BONAFIDE CERTIFICATE

Certified that this dissertation is the bonafide work of **Dr.N.MURUGAN** on **''COMPARISON OF A SINGLE SPOT CONCENTRATED SPUTUM AFB SMEAR VERSUS 2 DIRECT SPUTUM AFB SMEARS DONE REGULARLY IN REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME ''** during his MD (PULMONARY MEDICINE) course from April 2009 to April 2012 at INSTITUTE OF THORACIC MEDICINE AND RAJIV GANDHI GOVERNMENT GENERAL HOSPITAL – MADRAS MEDICAL COLLEGE, CHENNAI.

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DECLARATION BY THE SCHOLAR

I hereby declare that the dissertation entitled **"COMPARISON OF A SINGLE SPOT CONCENTRATED SPUTUM AFB SMEAR VERSUS 2 DIRECT SPUTUM AFB SMEARS DONE REGULARLY IN REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME"** submitted for the Degree of Doctor of Medicine in M.D, DEGREE EXAMINATION Branch XVII PULMONARY NEDICINE is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship, fellowship or similar other titles. It had not been submitted to any other university or institution for the award of any degree or diploma.

Place: Chennai
Date :

Signature of the scholar
Name : **Dr. N.MURUGAN.**

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CONTENTS

COMPARISON OF A SINGLE SPOT CONCENTRATED SPUTUM AFB SMEAR *VERSUS* 2 DIRECT SPUTUM AFB SMEARS DONE REGULARLY IN REVISED NATIONAL TUBERCULOSIS CONTROL PROGRAMME.

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BACKGROUND: Sputum smear microscopy is the backbone of Revised National tuberculosis control programme. It is an easy, safe and effective diagnostic method for pulmonary tuberculosis. But it involves three patient visits to the hospital till he collects the result. So we encounter a lot of initial defaulters on account of which we need a method to decrease the patient visit, without compromising on the results. So we compared Spot concentrated AFB Smear versus Spot and Home RNTCP AFB smear(2 Direct smears), and we found the additional diagnostic yield by concentrating spot and home RNTCP specimen and analyzed the results.

MATERIALS AND METHODS: A prospective study of 500 tuberculosis suspects, who came to the outpatient department of Institute of Thoracic Medicine & Govt.Rajiv Gandhi Generall Hospital ,Chennai. Persons who were not able to bring out sputum and individuals with hemoptysis were excluded from the study .All consenting participants were asked to give a good quality Spot and Early morning sputum of about 5ml, each of which was divided into 2 equal parts. Part 1 was subjected to concentration by adding 3% Ammonmium sulphate and 1%Sodium hydroxide and stained with Ziehl Neelson stain. Part 2 was smeared as per regular RNTCP technique .Chest X was taken for all the pulmonary tuberculosis suspects.

RESULTS : The results of 600 tuberculosis suspects were analyzed. RNTCP method diagnosed 77 cases. Concentration method diagnosed 79 cases. Single concentrated spot AFB smear itself diagnosed 78 cases, which is as good as RNTCP method. The difference in the yield between RNTCP method and Concentration method is statistically not significant. Statistically the level of agreement between the RNTCP method and the Concentration method is very good, calculated by kappa co-efficient.

CLINICAL IMPLICATION : In diagnosing pulmonary tuberculosis cases spot concentrated AFB Smear alone itself is as good as RNTCP spot and Home specimens combined. The number of visits required for diagnosing and initiating treatment is reduced. The problem of initial defaulters is eliminated.

BACKGROUND

William Osler once famously said “Everyone has a little Tuberculosis in us”. The burden of disease and death caused by tuberculosis is immense, with 8.8 million cases and nearly 2 million deaths estimated to have occurred in 2003 alone.[1] The HIV epidemic has had a huge impact, driving up incidence rates dramatically in sub-Saharan Africa.[1-4]In addition, tuberculosis is a major cause of death among people who are HIV infected, currently accounting for at least 11% of AIDS deaths worldwide.[5] An important barrier to global tuberculosis control is the low rate of case detection. Although the proportion of smear-positive cases identified at one end is increasing, the proportion that were identified globally under directly observed therapy (short course) programmes (the internationally recognised tuberculosis control strategy) was only 45% in 2003.[1]The World Health Assembly set a global target to detect 70% of new smear-positive cases (70% case detection rate) by 2005. This target was not met, on account of lack of co-ordination between public and private sector. [6] To prevent transmission of *Mycobacterium tuberculosis* and to provide appropriate care for patients, prompt and accurate diagnosis of tuberculosis is a matter of great urgency. [7, 8]

INTRODUCTION

Mycobacterium tuberculosis is the most successful pathogen to invade mankind, and the battle is still on, because of its evasive nature. Sputum direct smear microscopy is a valuable tool in this battle. Ever since the emergence of Revised National Tuberculosis Control Program, direct smear microscopy has been the backbone of it, the reason being, it is simple, widely available, it identifies the most infectious patients, cost effective, at the same time specific. It is a boon in low and middle income countries. The problem with direct smear microscopy is its varied sensitivity. This is because sensitivity depends upon collection of sufficient sputum, proper preparation of smears, good staining technique, careful examination of smears, and availability of a good quality microscope.[9] In developing countries the laboratory technicians sometimes tend to sidestep the sputum examination owing to the apprehensions about the infectiousness of the sputum samples and the cumbersome method of preparing direct smears from mucous portion of the sample. Smear-microscopy fails to diagnose patients who have low concentrations of *Mycobacterium tuberculosis* in their sputum, hampering tuberculosis control.[9]Conventional smear-microscopy involves smearing sputum on a microscope slide that is then stained and examined by high power microscopy to detect the causative acid-fast

bacillus *M. tuberculosis*. For a 50% probability of finding a single acid-fast bacillus in 100 microscopy fields, approximately 5,000 acid-fast bacilli must be present per ml of sputum.[10] Consequently the sensitivity of this technique is typically only 30-70% of the sensitivity of culture.[10]Tuberculosis patients who have AIDS and/or are children usually have lower concentrations of *M.tuberculosis* bacilli in their sputum, so the diagnostic sensitivity of smear-microscopy is lower in these patients.[11] Thus, reliance on smear-microscopy may cause missed or delayed tuberculosis diagnosis, potentially increasing morbidity, mortality and tuberculosis transmission. Increasing the sensitivity of tuberculosis diagnostic testing is a public health priority.[12]

When the number of visits made by the people increase, the number of drop outs during the diagnostic pathway also increase. Work in Malawi has shown that a significant (15%) proportion of smear positive patients attending a district hospital drop out of the diagnostic pathway before their results were communicated to them and treatment started.[13] Collection of multiple sputum samples on multiple days has the following disadvantages

(1)An increase in the lab work load that can reduce the accuracy of the results, with higher number of false positive and false negative interpretations,

(2)An increased burden on the patients because of the requirement for repeated health visits.

So any method which increases the performance of the smear microscopy, at the same time reduces the number of patient visits is going to have a huge public health impact. After a number of studies stressing the needlessness of doing the 3rd smear, [14] which has resulted in the number of smears done reduced to 2, we were curious to know whether it could still be reduced to a single smear, without compromising on the efficiency of the results obtained.

OBJECTIVE

1. To compare a single spot concentrated sputum AFB smear versus 2 direct sputum AFB smears done regularly in RNTCP (Spot & early morning specimen).

2. To find the additional yield in concentrating both the spot and early morning sputum specimen.

REVIEW OF THE LITERATURE

The primary mode of search for articles in this review was through internet databases like ‘pub med’, “Google scholar” and hand search of articles. The general search terms were ‘Mycobacterium tuberculosis’, ‘direct smear microscopy’, ‘sputum concentration methods’ & ‘optimization of smear microscopy’. The articles included in this review were those that primarily dealt with diagnosis of pulmonary tuberculosis through smear microscopy, various sputum processing methods, those that compared the sensitivity and specificity of the various sputum processing methods, those that compared the smear results with culture, those that dealt with the incremental yield of various sputum processing methods and those that dealt with the cost effectiveness and other economical issues. The studies that were excluded were

1. Articles in languages other than English for lack of comprehension

2. Those that included respiratory specimens other than sputum

3. Those that included *Atypical mycobacteria*

4. Those that that did not compare the direct smears with the processed smears.

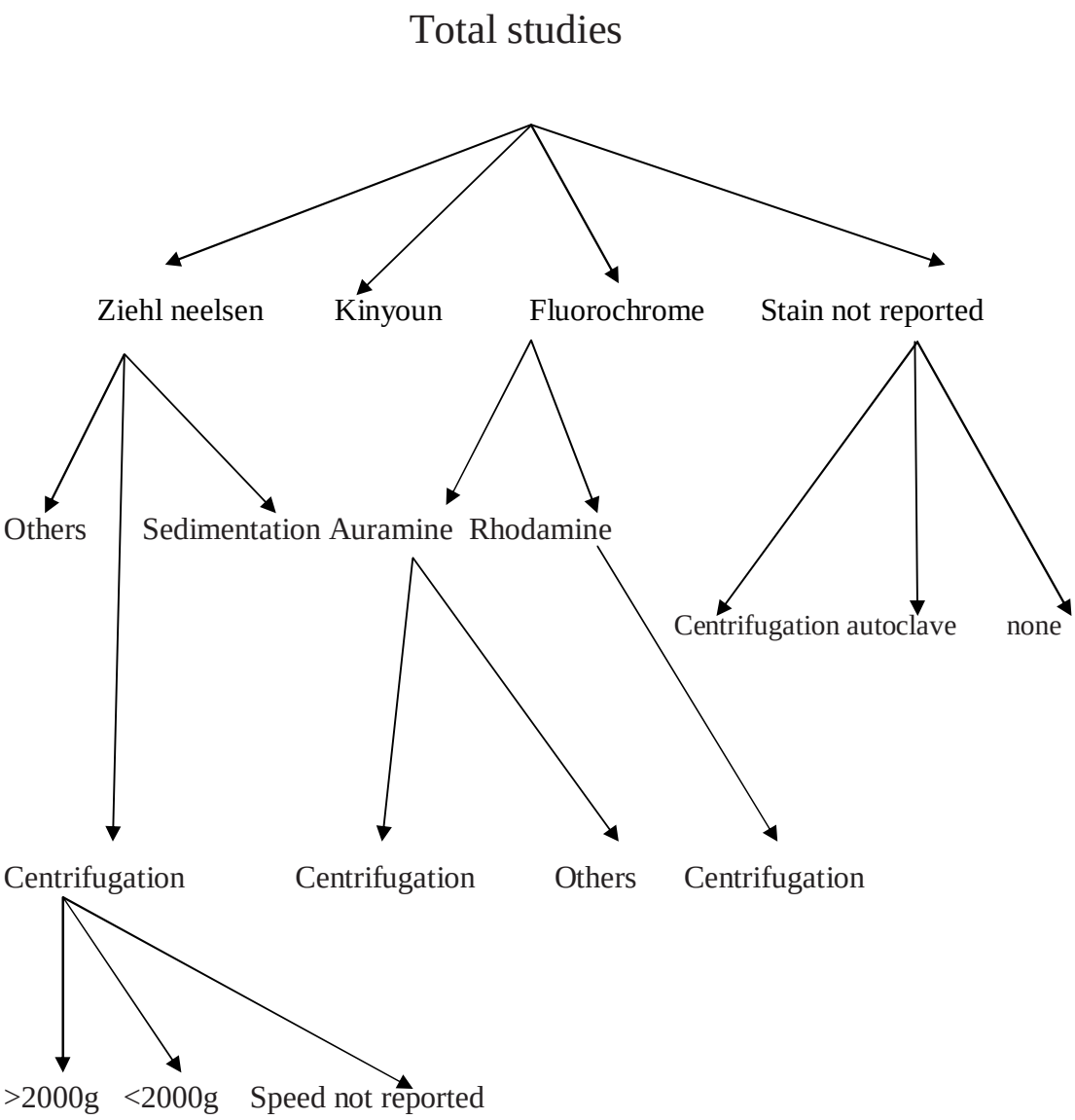
Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review

Karen R Steingart, Vivienne Ng, Megan Henry, Philip C Hopewell, Andrew Ramsay, Jane Cunningham, Richard Urbanczik, Mark D Perkins,Mohamed Abdel Aziz, Madhukar Pai.

This beautiful review article published in LANCET INFECTIOUS disease has dealt completely about the various sputum processing methods.

An attempt has been made to classify the various sputum processing methods based upon the stains used, physical or chemical processing methods, and the presence of culture. Further stratification has been done based upon the centrifugation by force (speed) less than 2000 g (<2500 rpm) and 2000 g or greater (≥2500 rpm); and studies that used gravity sedimentation by duration: short (≤ 1 h) and long (>1 h). An attempt has also been made for subgroup analysis of the sputum processing studies using Ziehl-Neelsen stain, using chemical and physical processing method. The main difference between these studies is the use of centrifugation and gravity dependent sedimentation method.

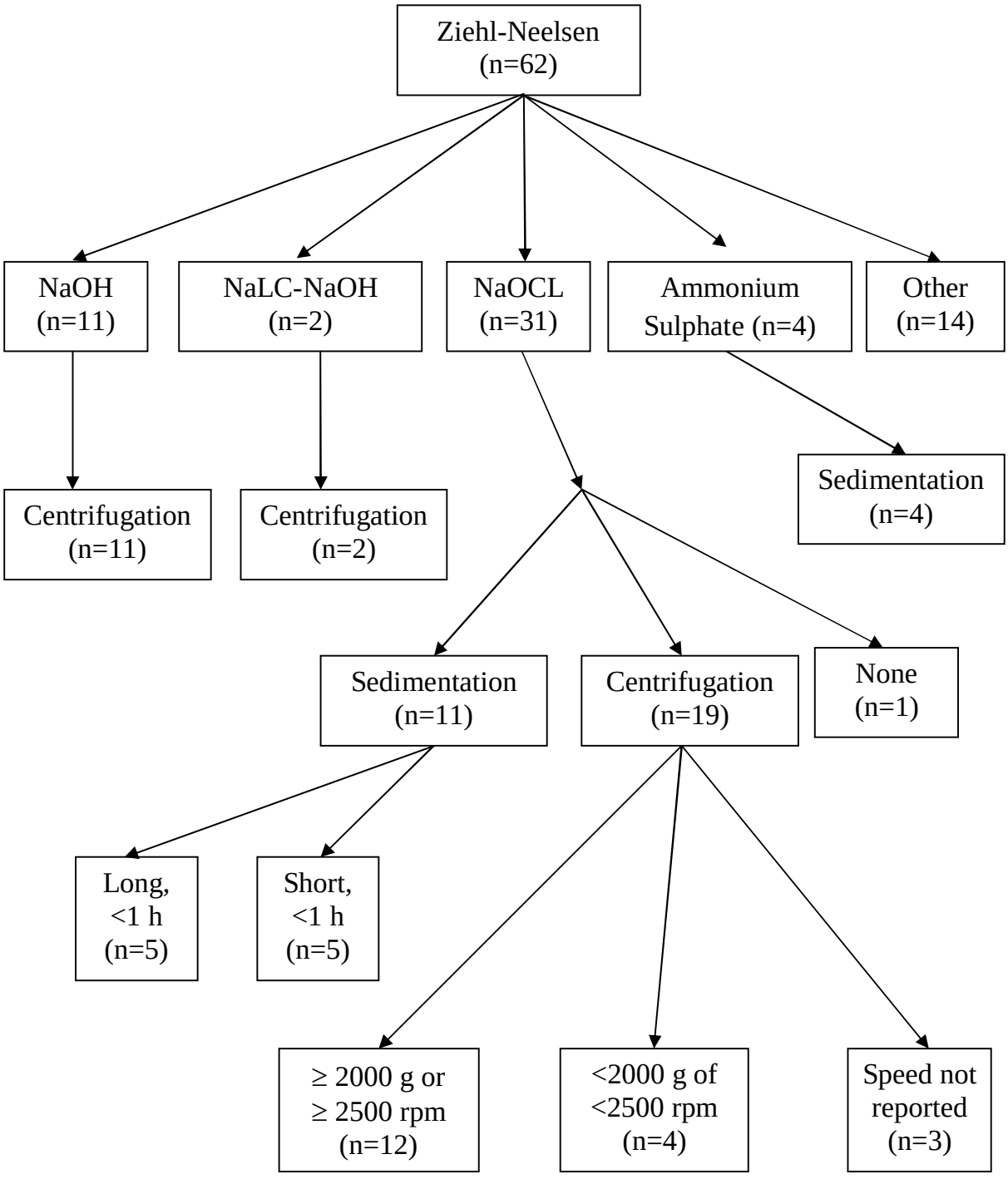
CLASSIFICATION TREE FOR SUBGROUP ANALYSES OF SPUTUM PROCESSING STUDIES, BY TYPE OF MICROSCOPY STAIN AND PHYSICAL METHOD.



Flowchart(1). **Classification tree for subgroup analyses of sputum processing studies, by type of microscopy stain and physical method.**

Some studies have also used centrifugation of sputum specimens without addition of any chemicals though without much yield. Although most of these studies are concerned with comparison of the direct smears with the processed smears, they are a heterogeneous group. Of the studies that have used Carbol fuschin as the primary stain, some have used Ziel-Neelsen while some have used Kinyoun staining, many studies have used different cut off for defining a positive smear, some studies have used only one sputum specimen to compare direct and the processed smears and various studies have used various time limits for reading a slide. There were also studies where there was no blinded interpretation of the slides.

CLASSIFICATION TREE FOR SUBGROUP ANALYSES OF SPUTUM PROCESSING STUDIES USING ZIEHL-NEELSEN STAIN, BY CHEMICAL AND PHYSICAL SPUTUM PROCESSING METHOD Flowchart (2).



CENTRIFUGATION WITH ANY CHEMICAL METHOD : TABLE -1

Study* (first author, year, country)	Study Popu-lation	Number patients or specimens	Chemical Processing method	Centri-fugation force/ speed	Centri-fugation time (min)	Sensitivity		Difference in sensitivity (PS-DS)
						DS	PS	
Allwood, 1997, Malaysia	PTS	173	NaOCl	1500 g	15	0.43 (0.29-0.58)	0.52 (0.38-0.66)	+9%
Angeby(b), 2000, Honduras	Routine sputum	303	NaOH	3000 g	15	0.57 (0.41-0.71)	0.76 (0.61-0.87)	+19%
Apers, 2003, Zimbabwe	PTS	256	NaOH	2000-3000 g	15-20	0.68 (0.61-0.74)	0.87 (0.82-0.91)	+19%
Bruchfeld (a),2000, Ethiopia	PTS	509	NaOCl	3000 g	15	0.54 (0.46-0.62)	0.63 (0.55-0.70)	+9%
Bruchfeld (b),2000, Ethiopia	PTS	96	NaOCl	3000 g	15	0.39 (0.29-0.49)	0.50 (0.40-0.60)	+11%
Chakravorty, 2005, India	PTS	571	USP	5000-6000 g	10-15	0.69 (0.63-0.74)	0.98 (0.96-0.99)	+29%
Farnia (a), 2002, Iran	PTS	430	NaLC-NaOH	3000 g	15	0.50 (0.38-0.62)	0.89 (0.79-0.95)	+39%
Gabre(a), 1995, Ethiopia	PTS	100	NaOCl	800-3000 g	15-20	0.31 (0.19-0.45)	0.69 (0.55-0.81)	+38%
Naganathan, 1979, India	PTS; abnormal chest radiograph	1499	NaOH	4000 pm	20	0.80 (0.77-0.84)	0.77 (0.74-0.81)	-3%
Perera, 1999, Sri Lanka	PTS	163	NaLC-NaOH	4000 g	15	0.63 (0.54-0.71)	0.92 (0.86-0.96)	+29%
Vasanthakumari (a), 1998, India	Symptomatic patients	1000	NaOH	3000 pm	15	0.57 (0.49-0.66)	0.91 (0.85-0.95)	+34%

CENTRIFUGATION WITH ANY CHEMICAL METHOD :

In the subgroup of studies with culture comparison, sputum processing yielded a mean 18% (95% CI 11–26%) increase in sensitivity, with 13 studies showing an increase, and one study showing a decrease. For 18 studies without culture, the mean increase in incremental yield after processing was7% (95% CI 3–11%), 15 studies reporting an increase, two studies reporting a decrease, and one study reporting no difference. In studies with culture comparison, the mean increase in sensitivity after culture comparison was 13% (95% CI -1-26).In all the studies the sensitivity of the processed smears were better than the direct smears. In studies without culture the mean increase in the incremental yield was 9% (95% CI 5-14).

GRAVITY SEDIMENTATION WITH ANY CHEMICAL METHOD.

16 studies, eight of which used culture, investigated the effect of sedimentation with various chemical agents, usually either bleach or ammonium sulphate. Of the eight studies with cultures, four used short sedimentation times of 30–45 min four studies used overnight sedimentation (table 2). In the subgroup of studies with culture, all four studies using overnight sedimentation found an increase in sensitivity,

GRAVITY SEDIMENTATION WITH ANY CHEMICAL METHOD : TABLE -2

Study* (first author, year, country)	Study Population	Number patients or specimens	Chemical Processing method	Sedimentation time	Sensitivity (95% Cl)		Difference in sensitivity (PS-DS)
					DS	PS	
Cuevas (a), 2005, Nigerial	PTS not on treatment	183	NaOCl	30-45 min	0.59 (0.52-0.66)	0.59 (0.52-0.67)	0%
Cuevas (b), 2005, Nigerial	PTS not on treatment	230	NaOCl	30-45 min	0.48 (0.42-0.55)	0.48 (0.42-0.55)	0%
Farnia (b), 2002, Iran	PTS	430	NaOCl	Overnight (12-15 h)	0.50 (0.38-0.62)	0.83 (0.73-0.91)	+33%
Farnia ©, 2002, Iran	PTS	430	Chitin	30 min	0.50 (0.38-0.62)	0.86 (0.76-0.93)	+36%
Garay, 2000, Zimbabwe	Symptomatic Patients	42	$(NH_4)_2SO_4$-NaOH	Overnight (12 h)	0.58 (0.43-0.71)	0.81 (0.68-0.90)	+23%
Lawson, 2006, Nigeria	PTS not on treatment	756	NaOCl	30-45 min	0.49 (0.44-0.54)	0.50 (0.45-0.54)	+1%
Selvakumar, 2002, India	Symptomatic Patients	2341	Phenol $(NH_4)_2SO_4$	Overnight	0.83 (0.80-0.86)	0.85 (0.82-0.88)	+2%
Vasanthakumari (b), 1988, India	Symptomatic Patients	1000	$(NH_4)_2SO_4$-NaOH	Overnight	0.57 (0.49-0.66)	0.91 (0.85-0.95)	+34%

with a mean gain of 23% (95% CI –1 to 47), whereas the four studies with short sedimentation times found a mean increase in sensitivity of 9% (95% CI –19 to 38). In the subgroup of studies without culture, all five studies using overnight sedimentation found an increase in incremental yield, with a mean gain of 5% (95% CI –3 to 14). An additional three studies with short sedimentation times without culture noted incremental yields of +5%, −4%, and +8%, respectively (table 3). 11 studies assessed sputum treatment with bleach and sedimentation. Of four studies that used culture, one study using overnight sedimentation noted a 33% increase in sensitivity, whereas the three studies with short sedimentation times reported no or little increase in sensitivity (table 2). Of seven studies without culture, four studies using overnight sedimentation showed a 6% mean increase in incremental yield (95% CI –6 to18), and three studies with short sedimentation times noted above had an inconsistent effect on incremental yield.

STUDIES COMPARING SENSITIVITY FOR ZIEHL-NEELSEN-STAINED DIRECT SMEARS AND SPUTUM SMEARS PROCESSED BY GRAVITY SEDIMENTATION WITH A CHEMICAL – TABLE 3

Study* (first author, year, country)	Number patients or specimens	Chemical Processing method	Sedimentation time	Positivity (95% CI)		Difference in sensitivity (PS-DS)
				DS	PS	
Contijo Filho (a), 1979, Brazil	122	NaOCl	30 min	0.34 (0.26-0.44)	0.30 (0.22-0.38)	-4%
Cuevas, 2005, Ethiopiat	198	NaOCl	30 min	0.26 (0.20-0.32)	0.31 (0.25-0.38)	+5%
Gebre-Selassie (a), 2003, Ethiopia	200	NaOCl	Overnight (16h)	0.09 (0.05-0.13)	0.26 (0.20-0.32)	+17%
Kochhar (b), 2002, India	1484	Ammonium Sulphate-NaOH	Overnight	0.09 (0.08-0.11)	0.13 (0.11-0.14)	+4%
Miorner (b), 1996, Ethiopia	545	NaOCl	Overnight (15-18 h)	0.17 (0.14-0.21)	0.21 (0.17-0.24)	+4%
Van Deun (a), 2000, Bangladesh	3287	NaOCl	Overnight	0.16 (0.14-0.17)	0.17 (0.16-0.18)	+1%
Van Deun (b) 2000, Bangladesh	1568	NaOCl	Overnight	0.16 (0.14-0.18)	0.17 (0.15-019)	+1%
Yassin, 2003, Ethiopia	200	NaOCl	30-45 min	0.18 (0.13-0.24)	0.26 (0.20-0.33)	+8%

SPUTUM PROCESSING METHODS FOR IDENTIFYING AFB IN HIV-INFECTED PATIENTS:

Although one study claims to have an increase in sensitivity of about 11%percent after sputum processing there are no conclusive data to say that in HIV infected patients' sputum processing definitely results in increase in sensitivity due to paucity of literature.

EFFECT OF PHYSICAL OR CHEMICAL SPUTUM PROCESSING METHODS ON SPECIFICITY OF SPUTUM SMEAR MICROSCOPY.

The specificity of microscopy after processing with physical and chemical methods was similar to the direct smear method. The mean specificity for direct smears was 0.98(range 0.92-1.00) and for processed smears 0.98(0.91-1.00).

Finally the review's conclusion was:

1. Sputum treated with bleach or sodium hydroxide and concentrated by centrifugation is, on average, more sensitive

2. Sputum subjected to overnight sedimentation preceded by treatment with Ammonium sulphate or bleach is, on average, more sensitive, based on a small number of studies

3. The specificity of the direct smears and the processed smears are the same

4. The gain obtained by processing sputum in HIV positive patients is equivocal.

There was one study which used centrifugation without chemical treatment. It reported a 7% increase in yield after processing with centrifugation of autoclaved sputum. The other chemical methods used for processing were chitin and N-acetyl cysteine & sodium hydroxide. Other physical methods were flocculation and floatation methods but the numbers of studies done were too small to comment on their efficacy.

Optimal tuberculosis case detection by direct smear microscope :How much better is more? Int J Tuberc Lung Dis 2002 ; 6:222-230.

Smear examination of two specimens for diagnosis of pulmonary tuberculosis in Tiruvallur district ,South India. Int J Tuberc Lung Dis 2004;8:824-828.

The above mentioned articles discuss the yield of morning versus spot specimens. The average yield of a single spot specimen was 73.9% compared with 86.4% for the average yield of a single morning specimen.

In the article "Concentrated smear microscopy :A simple approach to better case detection in Pulmonary tuberculosis, published in Indian Journal of Tuberculosis, R.Vasanthakumari et al discusses the advantage of Ammonium sulphate, gravity sedimentation method for processing the sputum specimen. The method is technically simpler than Petroff's method. Overnight deposition of sputum samples helps the bacilli to settle down and smears can be made from the deposits directly without any centrifugation. This makes the technique suitable for places with no electricity facilities. The use of cheap chemicals like Ammonium sulphate and Sodium hydroxide will make the procedure economically feasible for any peripheral health unit. This method does not require special skill and any para-medical personnel with a little training can carry it out with perfection. The solution used here for concentrating the sputum specimens is highly stable and retains its potency at room temperature indefinitely. Just like staining solutions this solutions can also be prepared at the district tuberculosis centers and supplied to the peripheral health institutions. As such this simple technique can be adopted for better case detection which is a key factor in tuberculosis control program.

Of late there has been much talk about bleach optimization of smear microscopy.

In the following article *"Improved sputum microscopy for a more sensitive diagnosis of Pulmonary tuberculosis", published in Int J of Tuberc Lung Dis in 2000,K.A.K Angeby et al* discusses the advantage of Sodium Hypochlorite, commonly called as house hold bleach, when it is utilized in the processing of sputum specimens. As a potent disinfectant, Sodium hypochlorite, kills mycobacteria and thus eliminates the risk of laboratory infection, a risk that cannot be neglected, especially in laboratories with inadequate safety standards. The technique was originally described in the 1940s, but has as far as we know, been almost totally forgotten. The hypochlorite method significantly increases the number of patients with pulmonary tuberculosis detected by microscopy at hospital level and somewhat less at peripheral health centers. The concentration of bacilli seen on each positive slide was highest with the Sodium hypochlorite method. It effectively kills *M.tuberculosis* , which makes the specimen safe to handle but of course, unsuitable for culture. The increased sensitivity is probably due to the greater concentration of AFB and to the fact that Sodium hypochlorite removes debris and leaves the microscopic field free for easy examination. It is inexpensive and available practically everywhere as household bleach. Moreover, the slides are easier and faster to read.

While we talk so much about various diagnostic modalities for the rapid diagnosis there is also an article which highlights *the judgment of the clinician in the diagnosis of pulmonary tuberculosis. In a review article published in Lancet Infect Dis 2003;3:288-96, Kamran Siddiqi et al* talks about the importance of the clinicians judgment in the diagnosis of Pulmonary tuberculosis.

Studies assessing clinical outcomes have shown more modest benefit with these techniques. In one study, the overall sensitivity increased from 54·2% to 63·1% after concentration ($p<0·0015$). In HIV-positive patients, sensitivity increased from 38·5% to 50·0% after concentration ($p<0·0034$). This improvement was less remarkable when compared with the sensitivity of direct microscopy supported by clinician's judgment in diagnosing pulmonary tuberculosis. So the clinician's judgment is always important because there is the problem of diagnosis of smear negative pulmonary tuberculosis. It has become urgent because the number of patients with pauci bacillary pulmonary tuberculosis in countries with HIV epidemic is increasing rapidly. It is going to be a challenge because of the atypical presentations of pulmonary tuberculosis in HIV infected patients.

MATERIALS AND METHODS

STUDY CENTRE : Outpatient department of Institute of Thoracic medicine,Chetpet and Rajiv Gandhi Government General Hospital, Chennai, Tamilnadu.

DURATION OF THE STUDY : February 2011-October 2011

STUDY DESIGN : Prospective observational study

SAMPLE SIZE: 600

INCLUSION CRITERIA:

Pulmonary tuberculosis suspects[15]

1. Individuals having cough with expectoration of 2 weeks or more
2. Contacts of smear-positive TB patients having cough with expectoration of any duration
3. Suspected /confirmed extra-pulmonary TB having cough with expectoration of any duration
4. HIV positive patient having cough with expectoration of any duration.

EXCLUSION CRITERIA:

1. Persons with haemoptysis

2. Persons unable to bring out sputum

3. Persons with prior history of tuberculosis treatment.

METHODOLOGY

600 participants who followed the above criteria were enrolled in the study. Pulmonary tuberculosis suspects who came to our outpatient department were clinically examined after a detailed history. After obtaining consent from them they were instructed how to give good quality sputum of about 5 ml and the RNTCP laboratory personnel collected the sputum containers. The sputum containers were labelled for identification and marked as spot specimen. Then they were given another cup for collecting the early morning sputum after detailed instruction on how to collect it. Then they were sent home after taking a Chest x ray. The spot specimen was divided into two equal halves. One half was subjected to direct smear method and the other half was subjected to concentration method. One day 2 the early morning specimen was obtained from the participants and labelled and marked as

early morning specimen. Again the sample was divided into two halves. One was subjected to direct smear method and the other was subjected to concentrated smear method. So from a single participant 4 smears were done. The results were documented.

CONCENTRATION TECHNIQUE

Attempts to improve the efficacy of sputum smear examination assume a greater significance in developing countries, where this method is extensively used for case detection. Direct smear is however inferior to the concentrated sputum techniques, the popular method being Petroff's technique. Unfortunately the technique is too cumbersome to be carried out under various field conditions.[15] So, a simpler technique that can be carried out in various field conditions was suggested by Dr.Vasanthakumari,[15] a time tested method that is being followed in our institution since 1988.The technique is described below.

Sputum sample is mixed with double the quantity of a solution containing 3% Ammonium Sulphate and 1% Sodium Hydroxide; the mixture is shaken well and left overnight at room temperature. Next morning, the supernatant fluid is decanted and smears made from the deposits. The sputum smears are then heat fixed and stained by Ziehl-

Neelsen method for Acid Fast Bacilli. Ammonium sulphate is an excellent homogeniser ;serves also as a fixative and makes the smears stick firmly to the slides.[16] Sodium hydroxide acts both as a homogeniser and as a sputolysin even at room temperature.AFB is detected microscopically in sputum direct smears and concentrated smears by employing bright field microscopy (1000 magnification).

ZIEHL-NEELSEN STAINING METHOD

A new unscratched slide was selected and labelled with the laboratory serial number using a diamond marking pencil. Smear was made from the yellow muco -purulent portion of the sputum sample using a loop. Smear was spread evenly, about 2 cm x 2 cm in size, was made neither too thick nor too thin. Then it was allowed to air dry for 15 to 30 minutes. The slide was then fixed by passing it over a flame 3 to 5 times, for 3 to 4 seconds each time. 1% Carbol fuchsin was poured to cover the entire slide. The slide was then gently warmed with the Carbol fuchsin on it, until vapours arose. Care was taken not to let it boil. Carbol fuschin was left on the slide for five minutes. Then the slide was rinsed with tap-water until all the free Carbol fuschin stain was washed away. 25% Sulphuric acid was then poured onto the slide for decolourising the primary stain. The slide was left to stand for 2 to 4

minutes. Then it was gently rinsed with tap-water, and then tilted to drain off the water.0.1% Methylene blue was poured onto the slide. The Methylene blue was then left on the slide for 30 seconds. The slide was then gently rinsed with tap-water and allowed to dry. The slide was then examined under the microscope using the 40x lens to select a suitable area and then examined under the 100x lens using a drop of immersion oil.[15] The grading of the positive smears are done by using RNTCP methodology.[15]

If the slide has:	**Fields**	**Grading**	**Result**
No AFB in 100 oil immersion fields	100	0	Negative
1-9 AFB per 100 oil immersion fields	100	Scanty	Positive
10-99 AFB per 100 oil immersion fields	100	1+	Positive
1-10 AFB per oil immersion field	50	2+	Positive
More than 10 AFB per oil immersion field	20	3+	Positive

Grading of the smears using RNTCP methodology. Table (4)

The direct smears and the concentrated smears were read by 2 separate laboratory technicians. They were double blinded and cross blinded to the results, to prevent them from influencing the results. The

experience of the lab technicians was 5&3 years respectively. The inter and intra reader reliability was assessed by the calculation of kappa coefficient, which measures the extent to which the results of both tests vary when read by two independent readers. A kappa value between 0.80 and 1 signifies almost perfect agreement.[14] External and internal quality assurance was maintained as usual during the period of study. External quality assurance was done in Pulianthope.

RESULTS

DEMOGRAPHY

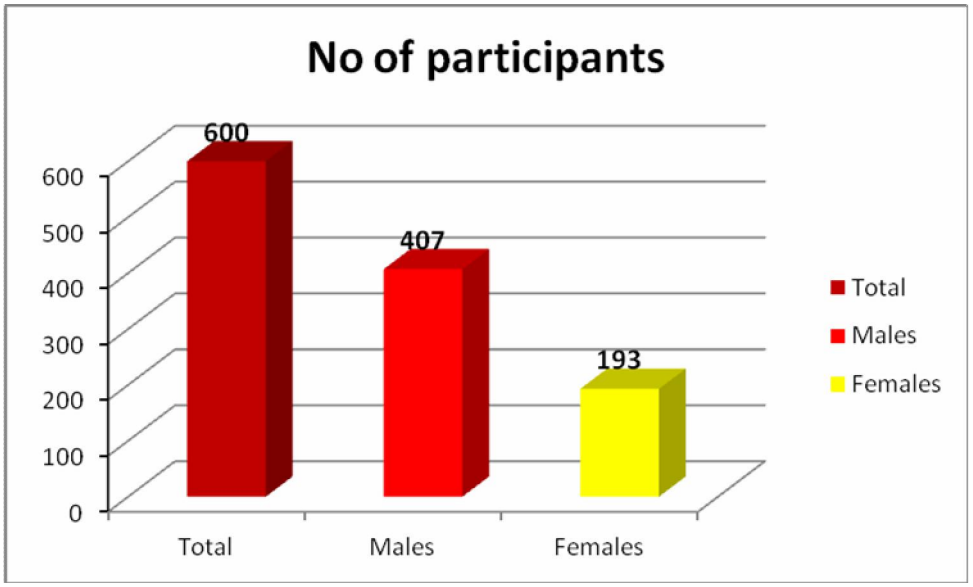
Total number of participants were 600.Among them 407 were males and 193 were females.Total number of pulmonary tuberculosis cases by direct smear method was 77.Total number of pulmonary tuberculosis cases by concentrated smear method was 79.During the diagnostic pathway 5 participants dropped out of the study on day 2.Among them one participant was sputum spot smear positive by both direct and concentration method.Since, from a single participant 4 smears were done,drop outs being 5,(4 *595=2390 smears) were analyzed.

	Male	**Female**
Total participants 600	407 (68%)	193 (32%)

Table (5).Table showing the number of male and female participants in the study .

A bar diagram was plotted to know the distributuion of male and female participants. The number of male participants were more. The

distribution is not comparable because of the method of the sampling technique employed,which is convenient sampling.



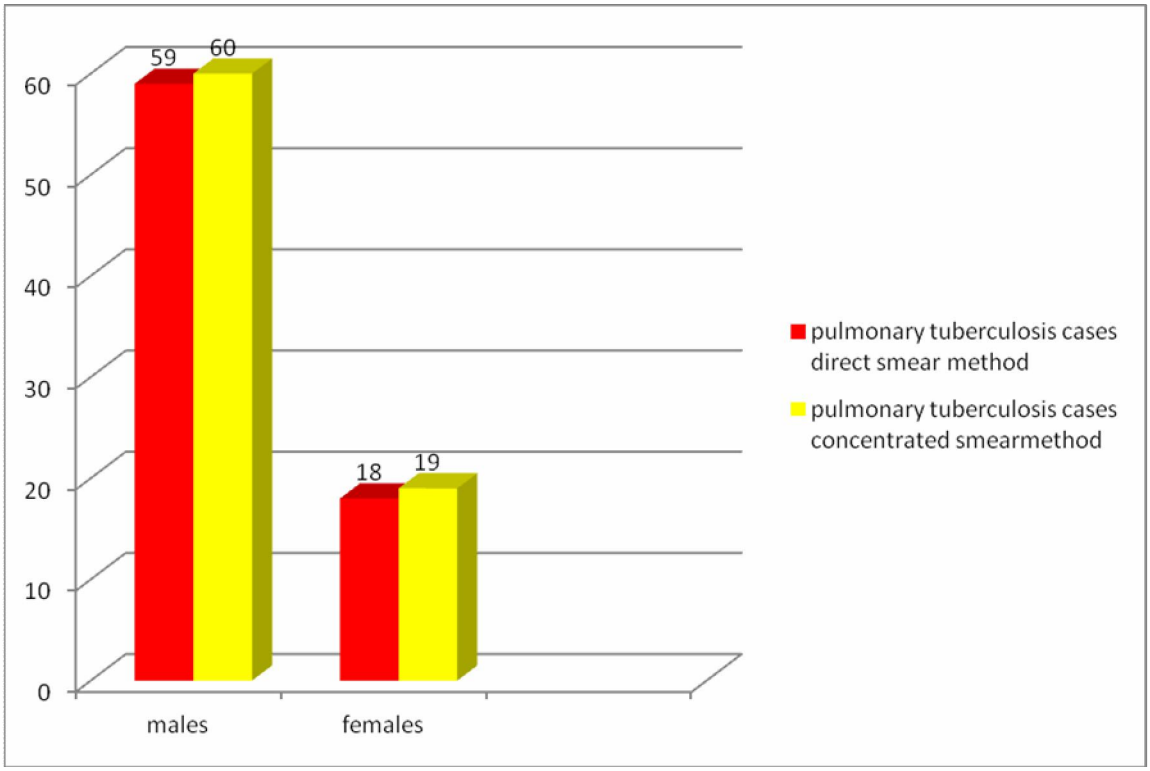
Bar diagram(1).the abscissae showing the sex of the participants and the ordinate showing the number of participants.

SEGREGATION OF MALE AND FEMALE PULMONARY TUBERCULOSIS CASES

According to the revised national tuberculosis control programme a a person is defined as a case of pulmonary tuberculosis even if one of the 2 smears is positive for acid fast bacilli.

		Male	Female
Total		407	193
Tuberculosis cases	Direct smear positive	59	18
Tuberculosis cases	Concentrated smear positive	60	19

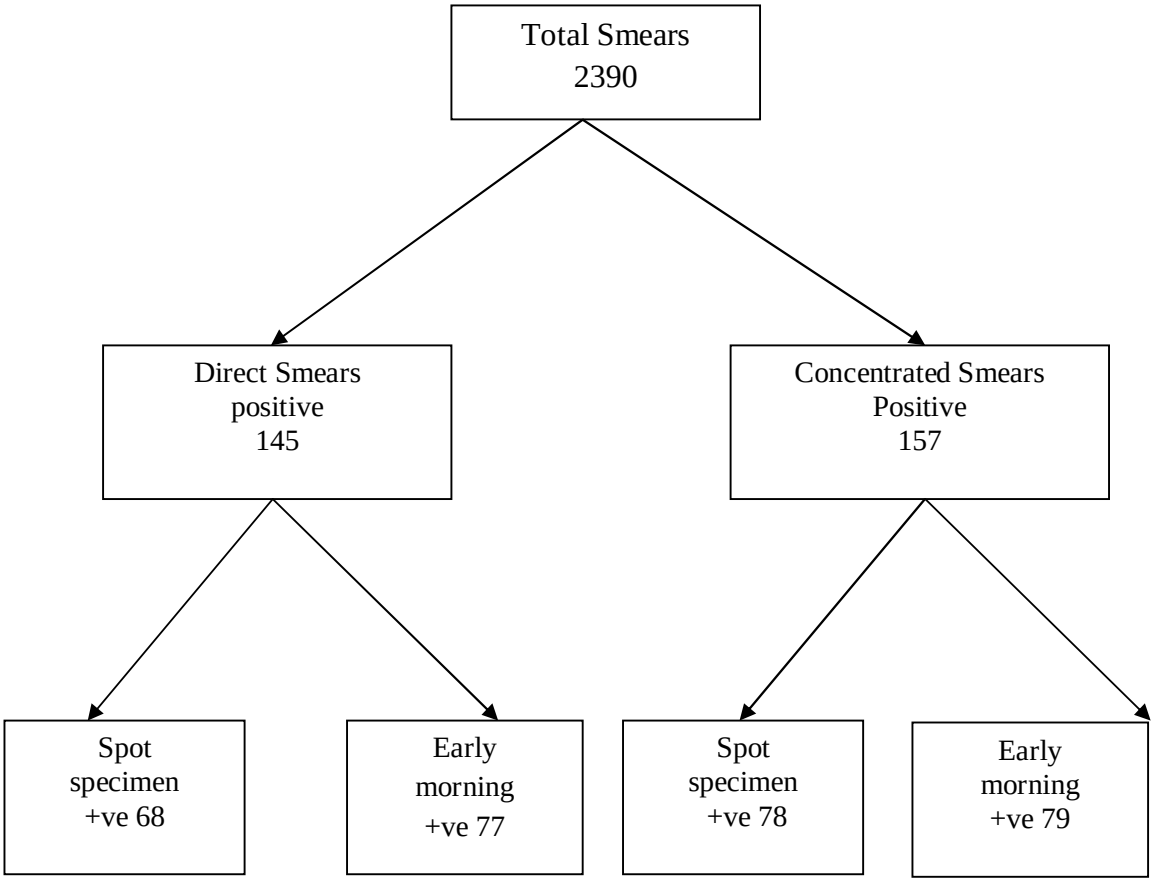
Table (6).In the above table(6) the total number of male and female pulmonary tuberculosis cases, and among them the number of male & female cases positive by direct and concentrated smear methods are tabulated.



Bar diagram(2).shows the number of male and female pulmonary tuberculosis cases poitive by direct and concentrated method.

Revised national tuberculosis control programme expects 5-15% of sputum positive patients among pulmonary tuberculosis suspects.In our study the percentage of of cases among the pulmonary tuberculosis suspects is 13% which is well within the RNTCP's expectation.

DISTRIBUTION OF POSITIVE SMEARS BY DIRECT AND CONCENTRATED METHOD ON DAY 1 AND DAY 2 EXPLAINED WITH THE HELP OF A FLOW CHART:

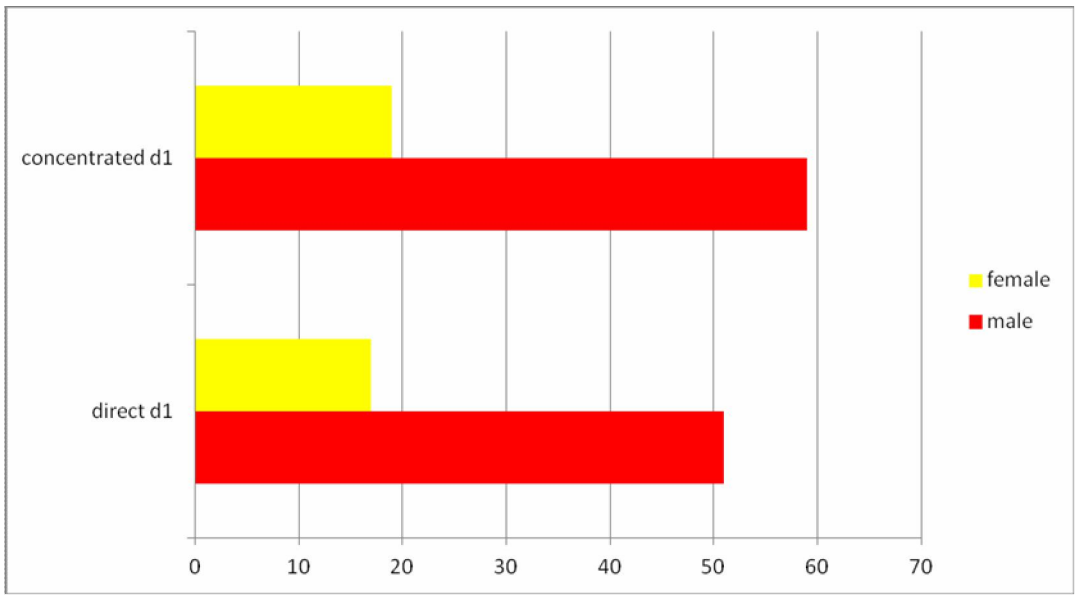


FLOW CHART (3) .Distribution of positive smears by direct and concentration on day 1 and day 2.

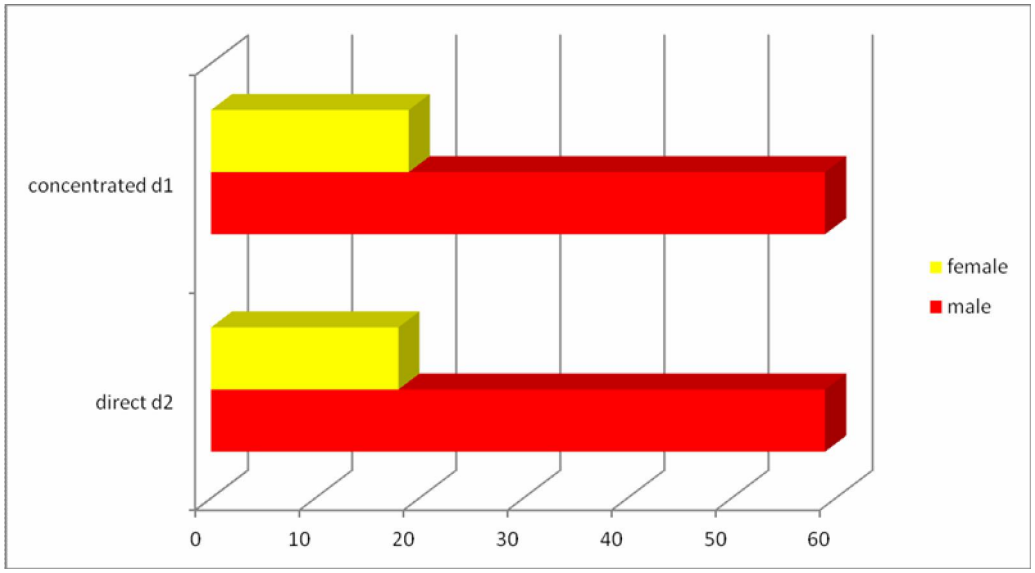
	Male	Female
Direct smear day 1	51	17
Direct smear day 2	59	18
Concentrated smear day 1	59	19
Concentrated smear day 2	60	19

Table(7). From the above flow chart and table we can conclude that grossly there is not much difference between the early morning direct smear and spot concentrated smear.

DEMOGRAPHIC DATA COMPARING MALE AND FEMALE CASES SHOWING THE SUPERIORITY OF SPOT CONCENTRATED SMEAR AGAINST SPOT DIRECT SMEAR.



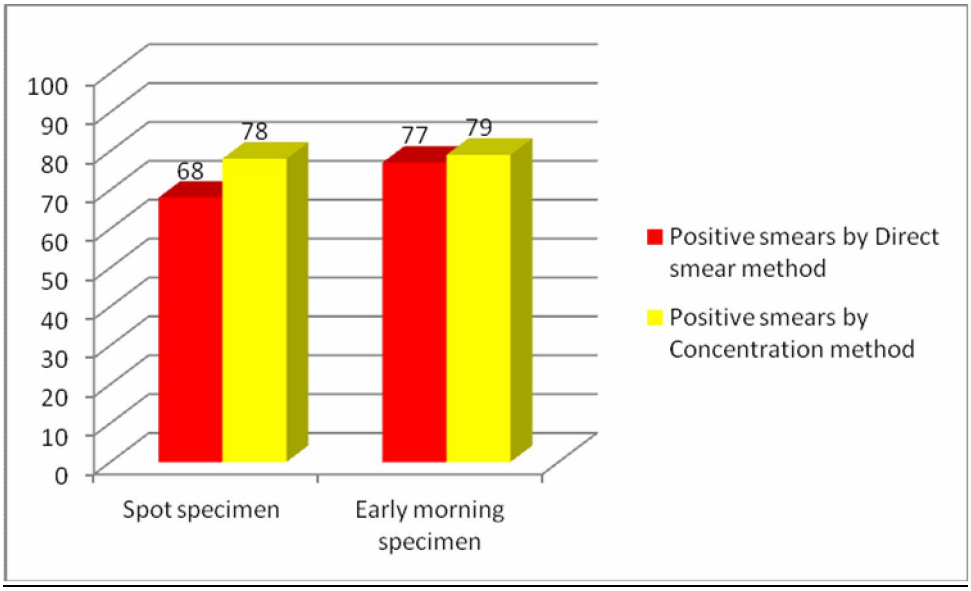
Bar diagram (3).Concentrated D1- concentrated AFB smear on spot, *directD1* direct AFB smear on spot.



Bar diagram (4).Concentrated D1- concentrated AFB smear on spot, *direct D2*-direct AFB smear on early morning.

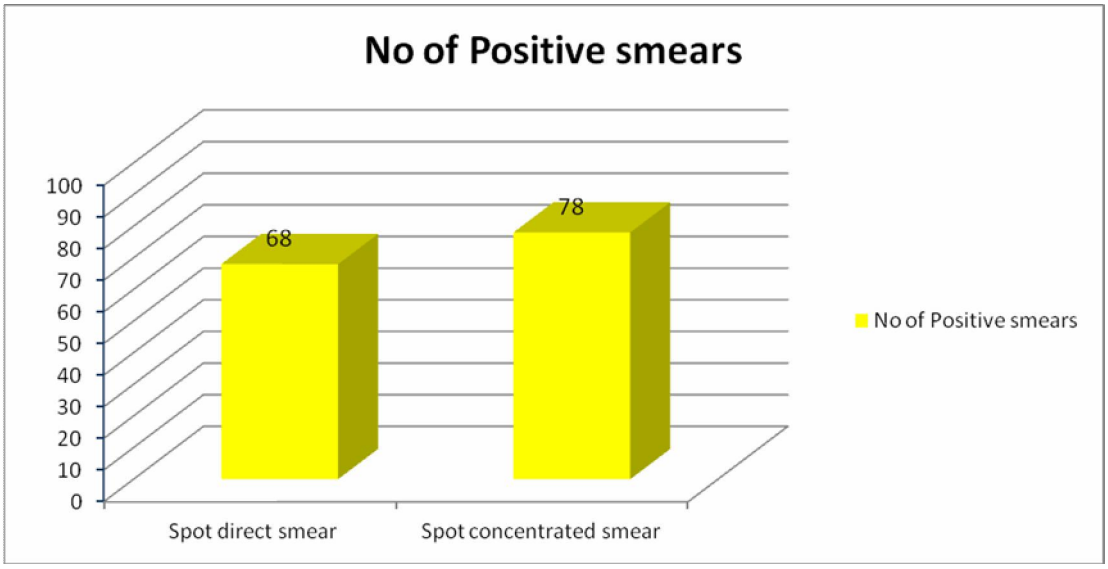
The above bar diagram shows the relative congruency between direct AFB smear on early morning and spot concentrated AFB smear.

COMPARISON OF CONCENTRATED AND DIRECT AFB SMEARS:



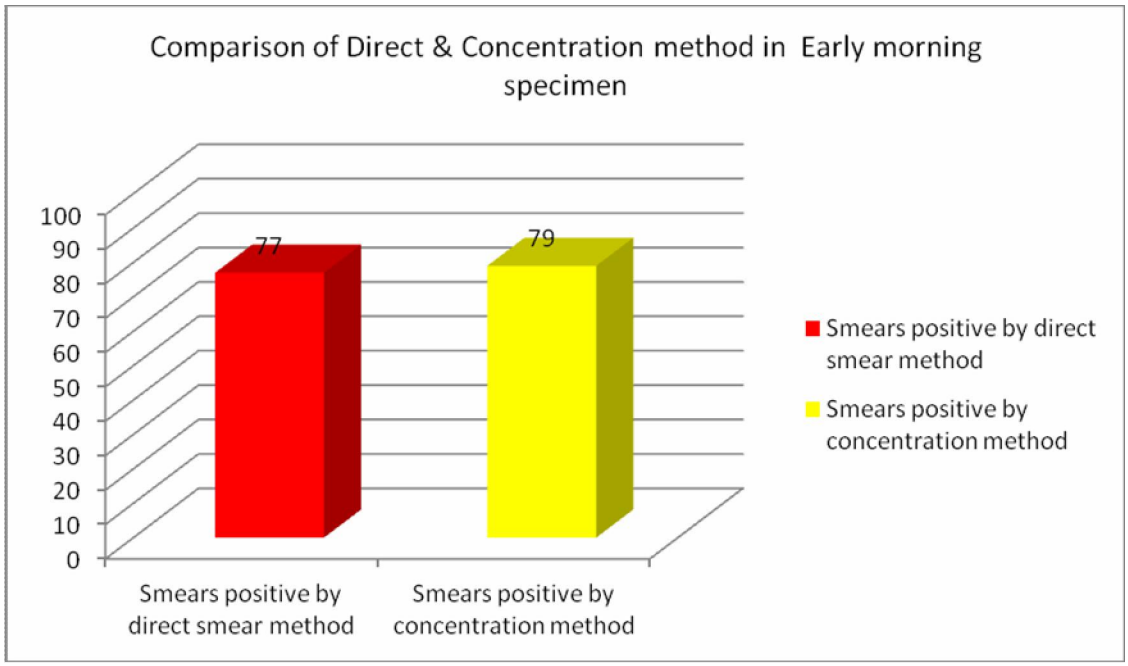
Bar diagram (5).The above bar diagram compares the results of direct and concentrated AFB smears on Day1 &Day 2.Here the number of direct smears positive on spot & early morning are 68 & 77 respectively. The number of concentrated smears positive on spot & early morning are 78 & 79 respectively.

COMPARISON OF SPOT DIRECT AND SPOT CONCENTRATED SMEARS :



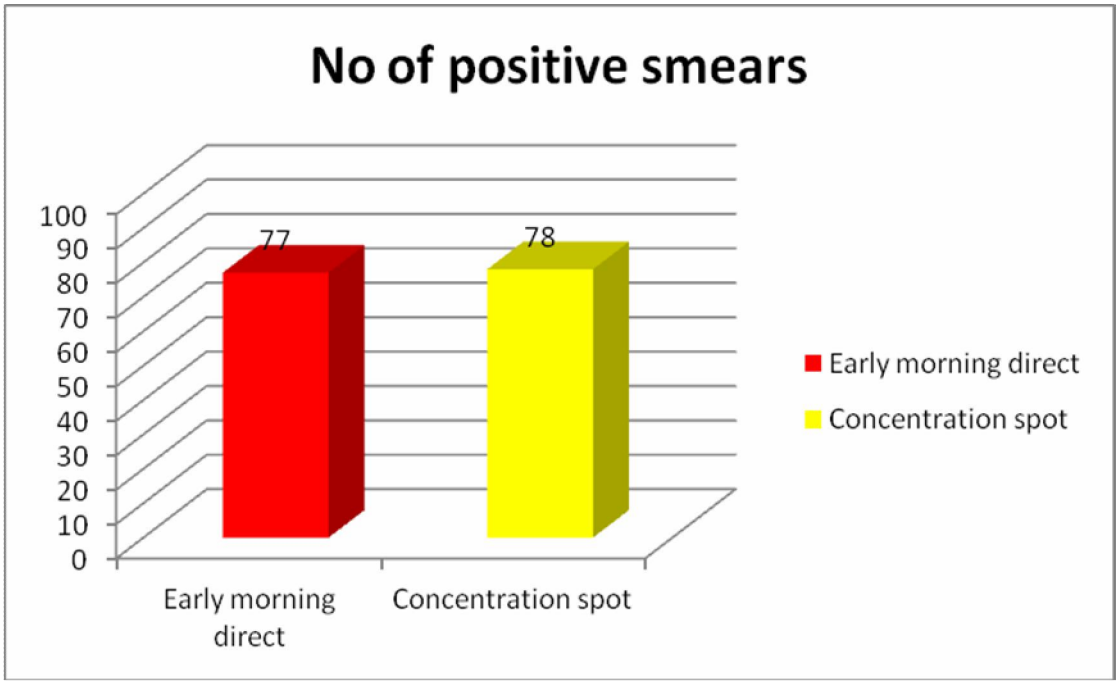
Bar diagram (6).The bar diagram clearly depicts the advantage of concentrating the Spot sputum specimen. There is a difference of 10 smears between spot concentrated and direct smears.

COMPARISON OF EARLY MORNING SPECIMENS OF DIRECT AND CONCENTRATED METHODS:



Bar diagram (7). In this comparison of early morning specimens of both direct and concentration method there is a difference of 2 smears by concentration method.

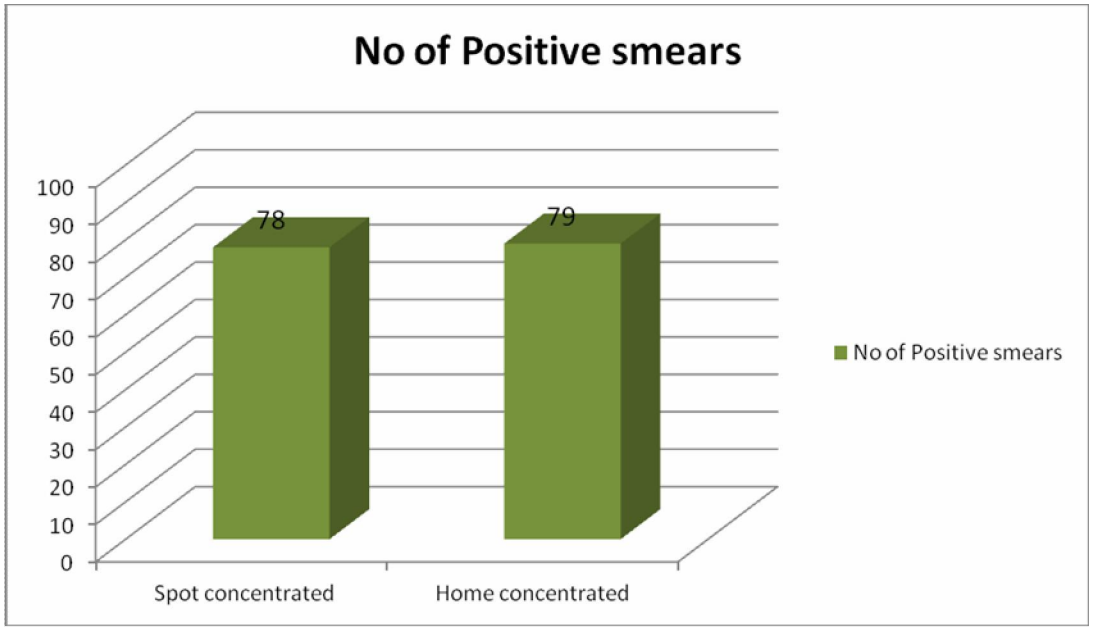
COMPARISON OF EARLY MORNING DIRECT AND SPOT CONCENTRATED AFB SMEARS



Bar diagram (8).

Although there isn't much difference, still spot concentrated AFB smear scores over early morning direct smear, widely regarded as the best because of the increased yield of bacilli in it.

COMPARISON OF SPOT AND EARLY MORNING CONCENTRATED AFB SMEAR:



The bar diagram clearly shows that by additionally concentrating early morning specimen the additional number of smears got is only 1 which is not statistically significant.

INCREASE IN THE YIELD OF POSITIVE SMEARS BY CONCENTRATION TECHNIQUE:

Total number of smears read = 2390

Proportion of smears positive by concentrated method

157 /2390 *100 = 6.6%

Proportion of smears positive by direct smear method

145/2390 * 100 = 6.0%

Increase in the yield is calculated as the Proportion of smears positive by concentrated method minus the Proportion of smears positive by direct smear method, which is only 0.5.

INCREASE IN THE YIELD OF ADDITIONAL NUMBER OF PULMONARY TUBERCULOSIS CASES BY CONCENTRATION TECHNIQUE:

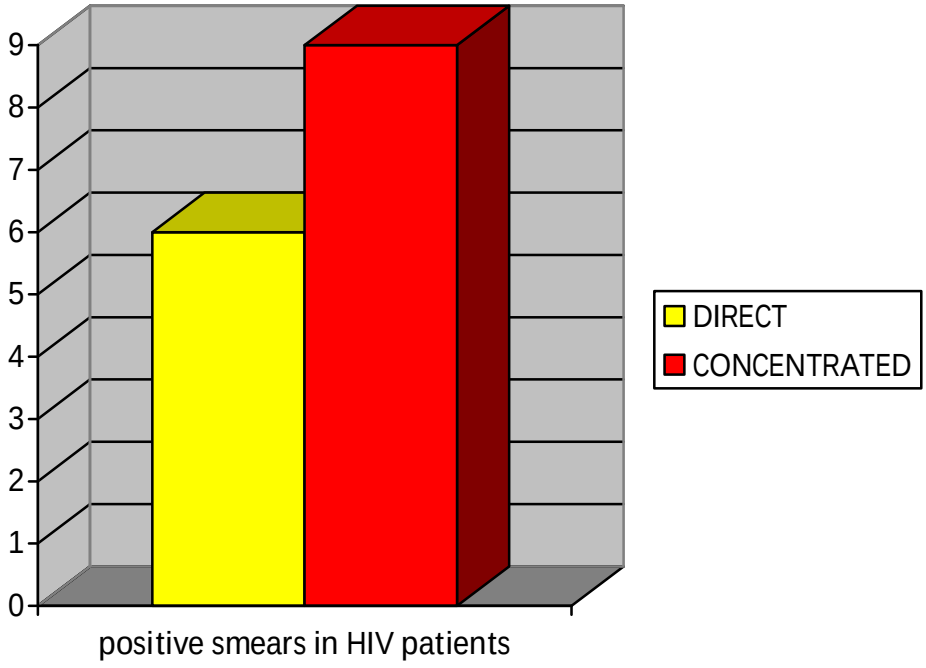
Total number of cases identified by concentrating spot as well as early morning sputum specimen is 79.

Total number of cases identified by a single concentrated spot sputum specimen is 78.

Total number of cases identified by direct smear method done by RNTCP protocol is 77.

YIELD OF CONCENTRATED AFB SMEAR IN HIV POSITIVE IN INDIVIDUALS*:*

There were totally 14 HIV positive patients .Among them 5 were sputum positive pulmonary tuberculosis cases. Among then 1 patient was diagnosed only with the help of concentration method alone.



In the above bar diagram the numbers of positive AFB smears are more in concentration method when compared with the direct smear method, although the increase is modest.

In HIV positive patients, the proportion of positive smears by concentration method minus the proportion of positive smears by direct

method (incremental yield) is equal to 20%. But the increased yield is not statistically significant.

CORRELATION OF CHEST SKIAGRAM OF PULMONARY TUBERCULOSIS SUSPECTS WITH SPUTUM AFB SMEARS:

Chest x ray	**Sputum negative**	**Sputum positive**	**Total cases**
0.NORMAL	462	2	464
1.CAVITY WITH INFILTRATES	3	36	39
2.INFILTRATES ONLY	11	35	46
3.MEDIATINAL ADENOPATHY	12		12
4.MEDIASTINAL ADENOPATHY WITH INFILTRATES	1		1
5.PLEURAL EFFUSION	3		3
6.MILIARY TUBERCULOSIS	1		1
7.CONSOLIDATION	2	1	3
8.LUNG ABSCESS	1		1
9.HEALED PULMONARY TUBERCULOSIS	4		4
10.CARDIOMEGALY	5		5
11.SOLITARY PULMONARY NODULE	1		1

Chest x ray	Sputum negative	Sputum positive	Total cases
12.BRONCHIECTASIS	7		7
13.HILAR PROMINENCE	2		2
14.BATWING APPEARANCE	1		1
15.DIAPHRAGMATIC EVANTERATION	1		1
2,5	2	1	3
1,5		1	1
2,3		2	2
2,3,5		1	1
3,5	1		1
1,3	1		1

Table (8) : From the above table it is clear that sputum positivity in smear microscopy correlates well with those chest skiagrams with cavity, infiltrates or both. There are 2 instances where chest skiagram is normal but sputum AFB smear is positive.

AGREEMENT BETWEEN THE CONCENTRATION AND THE DIRECT SMEAR METHOD:

Concentrated smear results	Direct smear results		
	Positive	**Negative**	**Total**
Positive	67	10	77
Negative	0	518	518
Total	67	528	595

Kappa statistics = 92% (the agreement between the two methods is very good)

(Tab.9) kappa co-efficient was calculated to know the agreement between spot concentrated and spot direct smear method.

Concentrated smear results	Direct smear results		
	Positive	**Negative**	**Total**
Positive	77	1	78
Negative	0	517	517
Total	77	518	595

Tab (10).Kappa statistics = 99%, (the agreement between the two methods is very good)

In (tab 10), kappa co-efficient was calculated to know the agreement between early morning concentrated and early morning direct smear method.

Kappa co-efficient is calculated to know the agreement between 2 qualitative data. When it is above 80% the agreement between the 2 data is very good. From the above 2 tables the agreement between the direct smear method and the concentrated method is well established.

COMPARISON OF THE GRADATION IN SPUTUM AFB SMEARS BETWEEN DIRECT AND CONCENTRATION METHOD:

Da * Ca Crosstabulation

Count

		Ca					
		0	1	2	3	4	Total
Da	0	518	9	0	0	1	528
	1	0	23	14	0	0	37
	2	0	0	10	2	0	12
	3	0	0	0	7	0	7
	4	0	8	0	0	3	11
Total		518	40	24	9	4	595

Da- spot direct smear, Ca-spot concentrated smear.0 No AFB,1 is 1+,2 is 2+,3 is 3+,4 is scanty.

9 smears classified as having no bacilli in direct method is 1+ in concentration method.8 smears noted as scanty is 1+,14 smears noted as 1+ is 2 +,2 smears noted as 2+ is 3+ ,1 smear noted as having no AFB scanty respectively in Concentration method.

Db * Cb Crosstabulation

Count

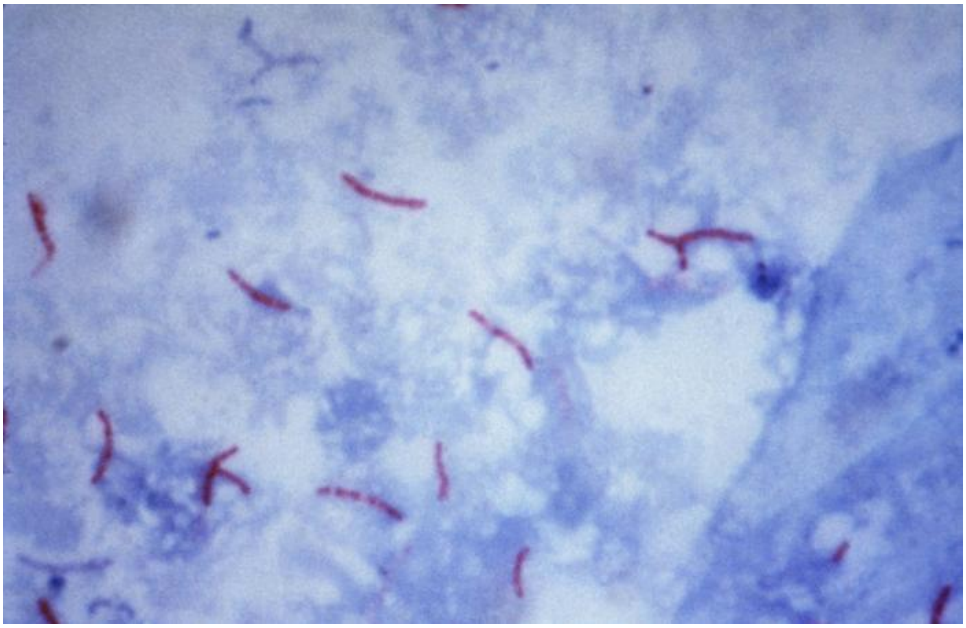
		Cb				
		0	1	2	3	Total
Db	0	517	1	0	0	518
	1	0	17	15	0	32
	2	0	0	15	8	23
	3	0	0	1	20	21
	4	0	1	0	0	1
Total		517	19	31	28	595

Db-early morning direct smear, Cb-early morning concentrated smear.0 No AFB, 1 is 1+, 2 is 2+, 3 is 3+, 4 is scanty.

In the table above 1 smear classified as 0, is 1+, 1 smear classified as scanty is 1+,15 smears classified as 1+ is 2+,8 smears classified as 2+ is 3+, respectively in concentration method. So when we concentrate sputum smear the probability of getting higher positive grade is more.

DISCUSSION

Prompt and accurate diagnoses, together with effective treatment are the essential elements of TB care and control. A confirmed diagnosis of pulmonary tuberculosis can only be established by isolating the *M. tuberculosis* complex or finding specific sequences of DNA in respiratory specimens.[18]



Ziehl- Neelsen stained Sputum AFB smear showing Acid fast bacilli.

However, because mycobacterium culture and molecular tests are complex and costly, they are not widely used in resource-poor settings where tuberculosis is prevalent, and sputum smear microscopy remains

the cornerstone of case detection. Optimization of smear microscopy techniques to improve the sensitivity and the development of new diagnostic tools are both areas of active investigation. In addition, existing recommendations regarding diagnosis are often not followed. A global situation assessment reported by the WHO suggested that delays in establishing a diagnosis are common.[1]This survey and other studies have also shown that clinicians, especially those who work in the private sector, often deviate from standard, internationally recommended diagnostic approaches.[20]

These deviations include under-utilization of sputum smear microscopy and over-reliance on radiography for diagnosis, as well as inappropriate utilization of poorly validated diagnostic tests, such as serologic assays. Thus, in addition to optimum technical utilization of sputum smear microscopy, educational and other efforts that foster utilization of appropriate diagnostic evaluations are essential.

Perusing the results, it is clear that a single spot concentrated smear is as good as 2 smears done by direct smear technique. While there is difference between spot & early morning direct smears, there is no major difference between spot & early morning concentrated smear results. There is no additional benefit in concentrating early morning

specimens, as the increase in the yield is not statistically significant. The total increase in the yield of concentrated method when compared to RNTCP method is 0.6%. While this increase in the yield is lower than other studies, where the average increase in the yield is around 7%, this can be explained. Ours is a tertiary care set up where the people approaches us as at an advanced stage, by the time, the bacterial load would be sufficient enough to be positive by direct smear microscopy.

Although the constraint of cost is present in concentration technique, the cost of doing 2 direct smears is always higher than doing single concentrated smear. When the number of patient visit is also brought into picture concentrated method would be much more economical. Thus the number of initial defaulters would be reduced, making a huge public health impact. By reducing the number of smears needed, patients could be moved from isolation earlier and more efficiently, and the cost of hospital stay could be reduced. The liquid ammonia method employed here is very economical as the chemicals used can be very cheaply procured.[16] This method of gravity sedimentation is as good as Petroffs method, which utilizes centrifugation .The deposits got after decantation gets fixed to the smear well in liquid ammonia method, on the contrary it doesn't in Petroff's

method. The time required to read a smear gets considerably reduced, here it is 7+/- 2min, whereas in direct smear method it is 10+/- 3min.[16]Although the results presented here are appealing these tests were done in laboratory conditions. How the concentration method behaves in field conditions must be further evaluated and the laboratory technicians must be sufficiently trained to get the expected results. The performance of any new diagnostic approach must be prospectively evaluated to determine the impact on case finding under program conditions.[18] Research will be required to evaluate the need for follow-up investigations of persons found to have Single spot concentration AFB smear negative. Research will also be needed to determine the optimum sampling strategy to improve case detection and get more patients into treatment programs. Particular attention must be given to the performance of a single specimen approach in HIV-infected persons.[18]

LIMITATION OF THE STUDY

Sputum culture was not done in this study on account of cost constraints. Since culture was not done, we were not able to determine sensitivity. We determined incremental yield. Incremental yield refers to the proportion of positive smears by the processed smear minus the

proportion of positive smears by the direct smear. Another limitation is the inherent bias of the laboratory technician. Though double and cross blinding were done to limit the bias, by the external appearance of the sputum smear one can easily identify the concentrated smears which could bias the technician.

IMPLICATION FOR FUTURE RESEARCH

Studies should be conducted in countries like India where the resources are poor, in settings that present a variety of epidemiological circumstances, especially in those with low and high HIV prevalence. When any diagnostic method is introduced it should be tested to see its impact on case finding under programmatic conditions. Research must be focussed on people who have clinical suspicion of tuberculosis with both the sputum specimens negative and optimal sputum processing methods to improve case detection and to enrol more patients into treatment program. Another problem is the deadly duo '*HIV and TUBERCULOSIS'*, where the diagnosis of tuberculosis by sputum microscopy becomes much more difficult on account of the decrease in the yield of AFB.[11] Operational research should address the issue of dropouts during diagnostic pathway and focus on the cost effectiveness and the logistics of implementing a single specimen strategy for TB case

finding, particularly in high prevalence resource poor settings. The cost of travelling and the accessibility of designated microscopy centres by the patients must be kept in mind.[18]

IMPLICATIONS FOR POLICY

1. The smear microscopy workload and the human resources available

2. The potential increase in the number of people dropping out of the diagnostic pathway

3. The potential decrease in the cost and time by optimising smear microscopy

4. The potential decrease in the number of smears required for blinded rechecking in quality assurance programs

5. The potential for increase or decrease in case detection

6. Strategies for following those patients who have two negative smears. [18]

CONCLUSION

1. A properly prepared single concentrated spot AFB smear is as good as 2 Direct AFB Smears done by RNTCP method.

2. The additional yield of sputum positive pulmonary tuberculosis cases by concentrating both the spot and early morning sputum specimen is 2, statistically insignificant.

3. The percentage of positive smears identity by Concentration method is 6.6 percent, in Direct smear method it is 6.0%, the additional yield being 0.6%, which is statistically insignificant.

4. The overall level of agreement between Direct smear method and Concentration method is above 95 percent. (kappa co-efficient greater than 80% is good agreement), which is very good agreement.

5. Though the difference between direct smear method and concentration method, in terms of results, is not much, the result obtained by 2 direct smears done over a period of 2 days is obtained by a single concentrated spot-sputum specimen.

6. As far as HIV positive patients with sputum positive pulmonary tuberculosis is concerned the yield is more in the concentration method when compared to direct smear method, but it is not statistically significant.

7. Single concentrated smear results in less number of patient visits to the hospital, so the number of dropouts during the diagnostic pathway is considerably reduced.

8. By doing a single concentrated smear workload of the laboratory personnel is considerably reduced.

9. Ammonium sulphate and sodium hydroxide used in the method is easily procurable and cheap, so the concern of cost is a little bit reduced.

10. When indirect costs made by the patient for transportation for 3 days, till he collects the results are considered, a single spot concentrated smear can be deemed economical.

11. The validity of the concentration method under field conditions should be studied. Proper training and motivation for the laboratory personnel must be given to acquire the desired results.

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PROFORMA

Name of the patient Age Sex Date

OP Number:

Presenting complaints :

1. cough with expectoration greater than 2 weeks

2. close contacts of sputum positive pulmonary tuberculosis patients

3. Extra pulmonary tuberculosis, confirmed/suspected, with cough of any duration

4. HIV positive patients with cough of any duration

5. Radiological suspicion of pulmonary tuberculosis

Chest x ray finding:

Sputum AFB results

DIRECT SPOT CONCENTRATION SPOT

DIRECT EARLY MORNING CONCENTRATION EARLY

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

Telephone No: 04425305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To

Dr. N. Murugan
PG in MD TB & RD
Madras Medical College, Chennai -3.

Dear Dr. N. Murugan

The Institutional Ethics Committee of Madras Medical College reviewed and discussed your application for approval of the proposal entitled "Comparison of spot and the next day sputum AFB semear versus spot concentrated AFB smear in pulmonary tuberculous suspects" No. 38022011.

The following members of Ethics Committee were present in the meeting held on 17.02.2011 conducted at Madras Medical College, Chennai -3.

1.	Prof. S.K. Rajan, MD	– Chairperson
2.	Prof. A. Sundaram, MD Dean i/c , Madras Medical College, Chennai -3	– Member Secretary
3.	Prof R. Sathianathan Director , Institute of Psychiatry, MMC,Ch-3	– Member
4.	Prof R. Nandhini, MD Director, Institute of Pharmacology, MMC, Ch-3	– Member
5.	Prof. Pregna B. Dolia MD Director , Institute of Biochemistry, MMC, Ch-3	– Member
6.	Prof. C. Rajendiran .MD Director , Institute of Internal Medicine, MMC, Ch-3	– Member
7.	Prof. Geetha Subramanian, MD,DM Prof. & Head , Dept. of Cardiology, MMC, Ch-3	– Member
8.	Thiru. A. Ulaganathan Administrative Officer, MMC, Chennai -3	– Layperson
9.	Thiru. S. Govindasamy . BA.BL	– Lawyer
10.	Tmt. Arnold Soulina	– Social Scientist

We approve the proposal to be conducted in its presented form.

Sd /. Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information / informed consent and asks to be provided a copy of the final report

Member Secretary, Ethics Committee

Information sheet

We are conducting a prospective study on **'COMPARISON OF SPOT AND NEXT DAY EARLY MORNING SPUTUM AFB SMEAR AND CONCENTRATED SPUTUM AFB SMEAR IN PULMONARY TUBERCULOSIS SUSPECTS"** in the department of Pulmonary medicine in government general hospital and Madras Medical College,Chennai.

The purpose of the study is to know the sensitivity,specificity,positive predictive value and negative predictive value of concentrated Sputum AFB smear when compared to RNTCP SPUTUM AFB SMEARS.

The privacy of the patient in the research will be maintained throughout the study.In the event of any publication or presentation resulting frorn the research,no personally identifiable information will be shared.

Taking part in this study is voluntary.You are free to decide whether to participate in this study or to withdraw at any time:your decision will not result in any loss of benefits to which you are otherwise entitled.

The results of this special study will be intimated to you at the end of the study or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator, **Signature of the participant,**

Date:

PATIENT CONSENT FORM

Study Details : "Comparison of Spot And The Next Day Sputum AFB Smear Versus Spot Concentrated AFB Smear In Pulmonary Tuberculous Suspects"

Study Centre : Department of Thoracic Medicine, Madras Medical College, Chennai.

Patient may check (✓) these boxes

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask question and all my questions and doubts have been answered to my complete satisfaction. ☐

I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected. ☐

I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study. ☐

I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any unexpected or unusual symptoms. ☐

I hereby give permission to undergo complete clinical examination and diagnostic tests including hematological, biochemical, radiological tests. ☐

I hereby consent to participate in this study. ☐

Signature/ Thumb Impression:

Patient Name and Address: Place Date

Signature of Investigator

Study Investigator's Name: Place Date

ஆராய்ச்சி தகவல் தாள்

சென்னை அரசு பொது மருத்துவமனையில் "திருத்தி அமைக்கப்பட்ட தேசிய காசநோய் தடுப்புத் திட்டத்தின் கீழ் செய்யப்படும் சளி பரிசோதனையையும், முதல்நாளே செய்யப்படும் திண்மையூட்டப்பட்ட சளி பரிசோதனையும் ஒப்பிடும் ஆராய்ச்சி நடைப்பெற்று வருகின்றது"

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். இந்த ஆராய்ச்சியில் பங்கேற்பதால் தங்களது நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்கு உள்ளாகாது என்பதையும் தெரிவித்துக் கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துகளை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிட மாட்டோம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியிலிருந்து பின் வாங்கலாம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

இந்த சிறப்புப் பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின்போது அல்லது ஆராய்ச்சியின் முடிவின்போது தங்களுக்கு அறிவிக்கப்படும் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம் **பங்கேற்பாளர் கையொப்பம்**

தேதி

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு

திருத்தி அமைக்கப்பட்ட தேசிய காசநோய் தடுப்புத் திட்டத்தின் கீழ் செய்யப்படும் சளி பரிசோதனையையும், முதல்நாளே செய்யப்படும் திண்மையூட்டப்பட்ட சளி பரிசோதனையும் ஒப்பிடும் ஆராய்ச்சி.

பெயர்	:	தேதி	:
வயது	:	உள்நோயாளி எண்	:
பால்	:	ஆராய்ச்சி சேர்க்கை எண்	:

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கம் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.

எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு நான் எனது சம்மதத்தை தருகிறேன். மற்றும் ஆராய்ச்சியில் பங்கேற்க நான் சம்மதம் தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்ப்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் நான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் நான் புரிந்து கொண்டேன்.

நான் என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக் கொள்ள சம்மதிக்கிறேன்.

கையொப்பம்